Life Sciences, Vol. 32, pp. 1547-1552
Printed in the U.S.A.

Pergamon Press

INFLUENCE OF CLONIDINE ON THE ACUTE DEPENDENCE RESPONSE ELICITED IN NAIVE RATS BY NALOXONE

Richard M. Eisenberg

University of Minnesota, Duluth
School of Medicine
Department of Pharmacology
Duluth, Minnesota 55812

(Received in final form December 21, 1982)

Summary

Clonidine has been used successfully in the treatment of opiate dependence. The discomforting effects of withdrawal are attenuated by the drug. The question of whether the more central process of dependence is affected by clonidine was tested in the present study. Change in plasma corticosterone was used as the indication of the stress of acute withdrawal from morphine. Conscious, unrestrained male rats showed a dose-related, though somewhat delayed, increase in plasma corticosterone after clonidine (0.01-0.1 mg/kg). The suggested mechanism for this effect involves presynaptic inhibition of noradrenergic neurons inhibiting CRF (corticotropin-releasing factor) release. Similar animals showed an elevation of plasma corticosterone after naloxone (0.4 mg/kg) was administered 3 hrs following a single morphine-priming (10 mg/kg). The naloxone-precipitated response was unaffected by clonidine (0.04 mg/kg). This dose of clonidine did not substitute for morphine-priming to produce the naloxone-precipitated response. The data suggests that clonidine elevated plasma corticosterone by an indirect mechanism. Further, the stress associated with acute withdrawal is unaffected by clonidine suggesting that the drug does not alter dependence development.

The use of clonidine (1-3) in ameliorating the discomfort of withdrawal has begun a new era in the treatment of opiate dependence. This drug, which appears to affect noradrenergic synapses in a complex way, prevents the signs of abstinence directly rather than indirectly by substituting for the narcotic at the opiate receptor (4-6). We have previously reported that dependence can be demonstrated by changes in plasma corticosterone in a matter of hours following a single exposure to an analgesic dose of morphine (7,8). The present report examines the question of whether clonidine reduces the stress of withdrawal or affects the development of acute dependence.

Methods

Male rats (Harlan Sprague/Dawley, Madison, WI) weighing 200-350 grams were used. They were maintained separately after surgery at a constant temperature (21°) and light cycle (on 06:00-20:00 hrs). Standard rat chow and water (containing tetracycline, 0.8 mg/ml) were provided ad libitum. Each animal was allowed to acclimate to the surroundings and routine for at least 5 days prior to surgery.

Experimental procedure: The four-day procedure began with the surgical placement of the catheter via the external jugular vein to the entrance of the right atrium as already described (8). This implantation and the utilization of individual

0024-3205/83/141547-06$03.00/0
Copyright (c) 1983 Pergamon Press Ltd.

sound-proofed, one-way vision boxes allows for the serial sampling of blood from conscious, unrestrained animals. In order to maintain catheter patency during the three day interval between surgery and blood sampling, catheters were flushed with 0.1-0.2 ml heparin/saline, 500U/ml. In addition, animals were placed in the experimental chambers daily for 1 - 2 hours during this interval in order that they could accommodate to the surroundings. On day 4, rats were placed and connected in the chambers and two hours were allowed to elapse before blood sampling began at 09:30 hrs. This allowed for stabilization of the plasma corticosterone levels after handling. Each 0.6 ml blood sample was withdrawn following the removal of the void volume of the sampling tube. To reduce the effects of blood loss involved with sequential sampling, the fluid volume was replaced by saline after the first sample. After each subsequent sampling of blood, the cellular fraction from the previous sample, resuspended in saline, was injected. The samples were then transferred to test tubes and centrifuged, and the plasma separated and stored at 0°. Plasma corticosterone was determined by a modification of the fluorometric method of Glick *et al.* (9). In our procedure, methylene chloride was substituted for chloroform in the extraction of corticosterone and the dilute sodium hydroxide wash was omitted.

Experimental drugs and statistical analysis: All drug solutions were made so that the fluid volume injected was 1.0 ml/kg b.w. Equivalent amounts of the saline vehicle were administered to controls. Doses were calculated as the salt. Statistical analysis was done using Student's *t*-test. Levels of significance are $p<0.05$. Areas under the curve were calculated using the trapazoidal method (23).

Results

Results in Table I show the effects of various doses of clonidine on plasma corticosterone. The drug or saline was injected after zero time. For statistical analysis, each drug-treated group was compared to the saline control at the respective time interval. There appears to be a modest stimulation of hormone level with the higher two doses which occurs no earlier than 30 min. The dose-related effect is more clearly demonstrable in Figure 1 showing a correlation between the dose of clonidine and the area under the plasma corticosterone response curve.

The second experiment was designed to learn whether clonidine alters the naloxone-induced plasma corticosterone three hours after morphine. These results are shown in Figure 2. Morphine (10 mg/kg, i.v.) was administered to two groups of rats after the first blood sample was withdrawn. After the 150 minutes sample, one group received saline and the other clonidine (0.4 mg/kg, i.v.). Naloxone (0.4 mg/kg, i.v.) was injected following the 180 minute sample. In both groups the characteristic hormone response to morphine was elicited after injection. Naloxone predictably produced an increase in plasma corticosterone (after 3 hrs) as we have previously reported. The treatment with clonidine 30 minutes prior to naloxone failed to alter the response to the antagonist. Previous results have shown that naloxone after saline-priming or saline after morphine-priming does not evoke a corticosterone increase (8).

The third experiment was an attempt to find if clonidine could substitute for morphine as the priming agent for the naloxone response. The dose of clonidine chosen produced a moderate elevation in hormone level, shown in Figure 3. Following naloxone after 180 minutes the plasma corticosterone level continued to decline in contrast to what was observed after morphine in Figure 2.

TABLE I

Effects of several doses of clonidine HCl on plasma corticosterone levels. *doses of clonidine HCl, in mg/kg, injected after 0 minutes, values are mean ± S.E. † = p < 0.05 compared to saline treatment at the respective time interval.

	minutes							
Treatment	0	15	30	60	120	180	240	N
Saline	6.5±1.5	8.2±2.0	11.1±2.2	9.1±1.8	10.6±4.4	7.9±1.9	8.4±2.0	7
Clonidine (0.01)*	5.4±0.5	6.5±2.3	13.1±3.5	15.9±2.7	16.1±4.4	8.9±1.4	8.1±2.0	8
(0.02)	9.4±2.3	14.8±3.1	8.4±1.5	17.2±3.4	16.4±1.4	9.2±1.5	8.5±2.5	7
(0.04)	8.5±1.1	9.8±2.8	11.2±2.6	21.1±3.5†	21.1±6.6	9.6±2.2	8.0±2.1	6
(0.10)	5.1±0.8	15.8±5.2	25.6±6.6†	15.6±2.9	20.0±7.8†	21.1±4.1†	13.6±2.9	6

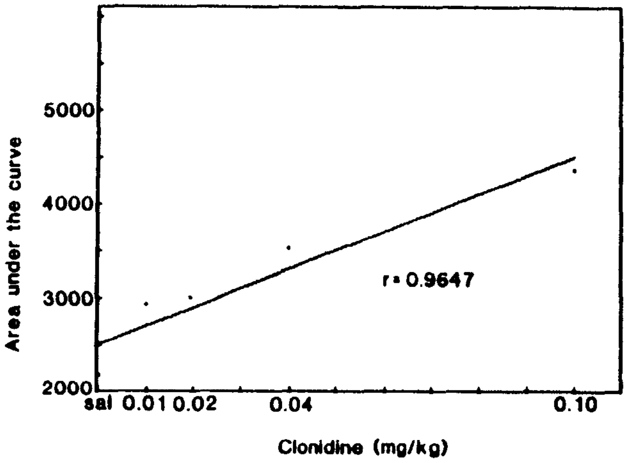

FIG. 1

Area under the plasma corticosterone dose-response curve for the results in Table I. Linear regression shows the correlation coefficient (r) for the dose of clonidine with increases in plasma corticosterone.

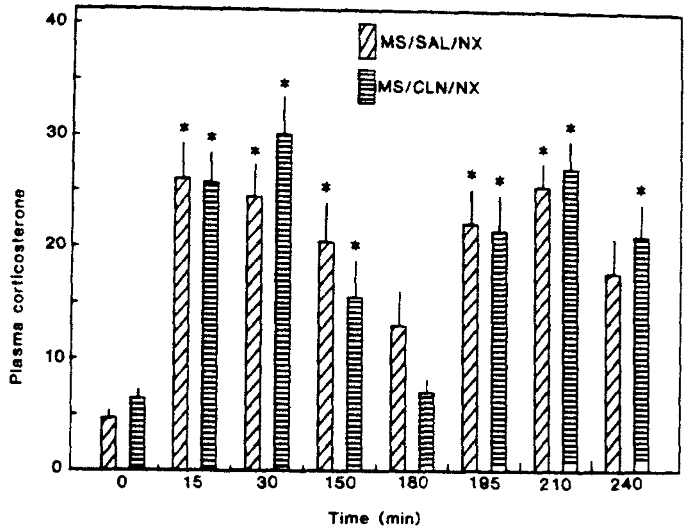

FIG. 2

Effects of clonidine (CLN) on the naloxone (NX)-induced elevation of plasma corticosterone (mean + S.E. in mcg/dl) 3 hrs after morphine (MS)-priming. *$p < 0.05$, compared to controls (0 and 180 minutes were controls groups for each subset). Numbers per group: MS/SAL/NX, 8; MS/CLN/NX, 9.

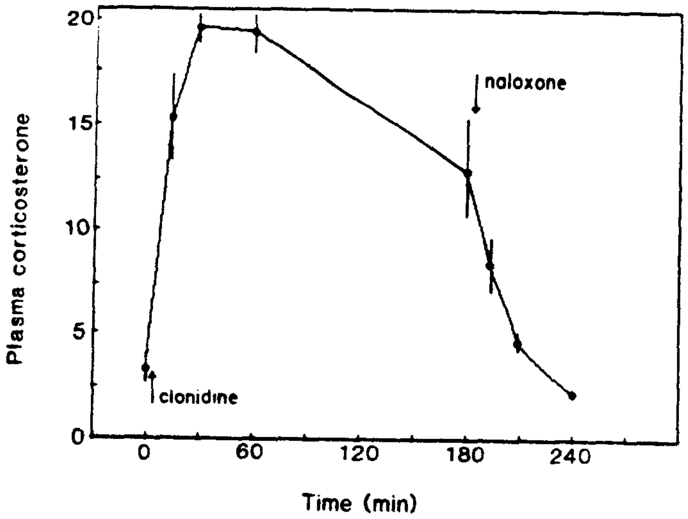

FIG. 3

Effects of naloxone on plasma corticosterone (mean ± S.E. in mcg/dl) 3 hrs after clonidine-priming. Number per group is 8.

Changes in
of narcotics
of the with
the priming
response (1(
withdrawal p

Before testi
hypothalamo-
be determin
elevation of
neurotransm
that choliner
contrast, a
during stres
clonidine is
facilitation
keeping wit
the central
release of
alpha$_2$ recep
effect of cl
(15) reporte
little change
or stress co
(18).

Our results
development.
not altered
agent to ini
manifestatio
making the dr
clonidine has
to naloxone-p
significantl
attenuated t
indication o
signs of with
corticostero
development is

The author w
Miss. Mary Wil
02015.

1. M.S. GO
2. M.S. GO
3. M.S. GO
A Study

Discussion

Changes in plasma corticosterone has proved to be a sensitive and reliable indication of narcotics withdrawal after either acute or chronic treatment (7,8). The magnitude of the withdrawal response, in the case of acute dependence, is directly related to the priming dose of opiate as well as the dose of naloxone used to precipitate the response (10). With this model we examined the question of whether clonidine prevents withdrawal *per se* or merely obtunds the undesirable symptomatology.

Before testing the effects of clonidine on this system, the effects of the drug on the hypothalamo-pituitary-adrenal axis, as demonstrated by plasma corticosterone, had to be determined. The results show that there is a dose-related, if somewhat delayed, elevation of this hormone (Table I). The latency suggests an indirect influence on neurotransmitters which control this neuroendocrine axis. It has been substantiated that cholinergic and serotonergic pathways facilitate the release of CRF (11-13). In contrast, alpha-noradrenergic pathways balance the system by inhibition, particularly during stress-induced stimulation (14). We suggest the increase observed after clonidine is the result of a depression of noradrenergic inhibition resulting in a net facilitation of the system and an elevation in plasma corticosterone. This is in keeping with the proposed mechanism of action of clonidine whereby the drug acts in the central nervous system on presynaptic alpha-adrenergic receptors to depress release of norepinephrine (4-6). The possibility that clonidine directly stimulates alpha$_2$ receptors to promote CRF release can not be excluded. Previous reports on the effect of clonidine on plasma corticosterone are somewhat inconsistent. Ganong *et al.* (15) reported an inhibition of stress-induced corticoid elevations in the dog but little change in basal level (16). Others have found no effect in the rat under basal or stress conditions (17) but an increase 45-90 minutes after infusion in dairy cattle (18).

Our results strongly suggest that clonidine does not affect the process of dependence development. The corticosterone stress response which is indicative of withdrawal is not altered by clonidine nor does this agent substitute for morphine as the priming agent to initiate acute dependence. The main action of the drug is on the manifestations of withdrawal. The characteristic discomfort in humans is ameliorated, making the drug useful in the treatment of opiate dependence (1-3). In the laboratory clonidine has also been shown to reduce withdrawal signs in animals. When given prior to naloxone-precipitated withdrawal, shaking, escape attempts, and diarrhea are significantly reduced in the rat (19-21). Sparber & Meyer (22) found that clonidine attenuated the naloxone-induced disruption in operant behavior (a behavioral indication of withdrawal) in opiate-dependent rats. Thus, even though behavioral signs of withdrawal are reduced in the presence of clonidine, persistent plasma corticosterone elevation after naloxone suggests that the basic process of dependence development is not altered by clonidine.

Acknowledgments

The author wishes to express his appreciation for technical assistance provided by Miss. Mary Wilkinson and Mrs. Judy Ostern. This study was supported by USPHS grant DA 02015.

References

1. M.S. GOLD, D.E. REDMOND, JR. and H.D. KLEBER, Lancet 1:929, (1978a).
2. M.S. GOLD, D.E. REDMOND, JR. and H.D. KLEBER, Lancet 2:599-602, (1978b).
3. M.S. GOLD and H.D. KLEBER, *Clinical Utility of Clonidine in Opiate Withdrawal: A Study of 100 Patients*, Alan R. Liss, Inc. New York, (1981).

4. N.E. ANDEN, H. CORRODI, K. FUXE, B. HOKFELT, T. HOKFELT, C. RYDIN and T. SVENSSON, Life Sci. 9:513-523, (1970).
5. K. STARKE, Naunyn Schmiedeberg's Arch. Pharmacol. 275:11-23, (1972).
6. T.H. SVENSSON, B.S. BUNNY and G.K. AGHAJANIAN, Brain Res. 92:291-306, (1975).
7. S.B. SPARBER, V.F. GELLERT, L. LICHTBLAU, and R.M. EISENBERG, Symposium on Factors Affecting the Action of Narcotics, Eds. Adler, Manara, and Samarin. Raven Press. New York, (1978).
8. R.M. EISENBERG and S.B. SPARBER, J. Pharmacol. Exp. Ther. 211:364-369, (1979).
9. D. GLICK, D.V. REDLICH and S. LEVINE, Endocrinology 74:653-655, (1964).
10. R.M. EISENBERG, Life Sci. (1982, in press).
11. G.A. HEDGE and P.G. SMELIK, Science 159:891, (1968).
12. E.V. NAUMENKO, Brain Res. 11:1, (1968).
13. M.T. JONES, E.W. HILLHOUSE and J. BURDEN, J. Endocr. 69:1-10, (1976).
14. R.I. WEINER and W.F. GANONG, Physiol. Rev. 58:905-976, (1978).
15. W.F. GANONG, N. KRAMER, J. SALMON, I.A. REID, R. LOVINGER, U. SCAPEGNINI, A.T. BORYCZKA and R. SHACKELFORD, Neurosciences 1:167-174, (1976).
16. F.J. HOLLAND, G.E. RICHARDS, S.L. KAPLAN, W.F. GANONG and M.M. GRUMBACH, Endocrinology 102:1452-1457, (1978).
17. D. JEZOVA-REPCEKOVA, M. VIGAS and A. KULIFAJOVA, Endocrinol. Experimentalis 12:3-8, (1978).
18. R.C. GOREWIT, J. Endocrinol. Invest. 4:135, (1981).
19. L. TSENG, H.H. LOH and E.T. WEI, Eur. J. Pharmacol. 30:93-99, (1975).
20. S. FIELDING, J. WILKER, M. HYNES, M. SZEWCZAK, W. NOVICK, JR. and H. LAL, J. Pharmacol. Exp. Ther. 207:899-905, (1978).
21. L. CERVO, C. ROCHAT, S. ROMANDINI and R. SAMANIN, Psychopharmacology 74:271-274, (1981).
22. S.B. SPARBER and D.R. MEYER, Pharmacol. Biochem. and Behav. 9:319-325, (1977).
23. R.J. TALLARIDA and R.B. MURRAY, Manual of Pharmacologic Calculations with Computer Programs, Springer-Verlag, New York, 1981.